REMARKS

Claims 23-38 currently appear in this application. Support for claims 34-38 can be found in the specification as filed at page 8, paragraphs 0032-0033. Support for claim 38 can be found in the specification as filed at page 10, paragraph 0036.

The Office Action of July 14, 2004, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

The Invention

The present inventor has discovered that certain fungi produce hemolysins which are specific to the particular fungus producing the hemolysin, and that these hemolysins or antibodies thereto can be measured in serum or other bodily fluids, or in samples taken from buildings. The present inventor for the first time has discovered how to measure or monitor exposure to fungi (molds) using the fungal hemolysins.

Present methods for measuring exposure to fungi are non-specific. For example, the antibodies produced against the spore coat proteins or mycelia of fungi are not unique to

specific fungi. Hemolysins, on the other hand, are unique, secretory proteins that are only released into an animal following environmental exposure by inhalation of the fungus. Hemolysins are not surface proteins, *i.e.*, they cannot be detected on the surface or on the outside of the spore or mycelium. Prior art workers tried to develop methods for detecting fungi by their surface components, but to date there has been no test which is specific for each individual fungus.

In other words, one must "open up" the cells to release the hemolysin(s). There are many conventional methods for doing this, all of which are well known to microbiologists, particularly since there is already available a commercial kit for detecting hemolysin of meningitis bacteria in samples. One way to obtain the hemolysin is to place the cells into a culture medium and allow the cells to secrete the hemolysins as they grow. Another method is to place the cells in a lysing solution. The lysing solution can comprise enzymes and other chemicals that help to open cells. These processes, however, can be slow. The simplest way is to place the sample into a "beat beating" tube and mechanically shatter the cells and spill the hemolysins. This can be effected in a matter of seconds, and is a technique well known to microbiologists. Submitted herewith is a coyp of ***JOEM*45(6)**: 591 (2003) listing many articles dealing with

assaying for bacterial hemolysins, as further evidence that one skilled in the art could readily detect the fungal hemolysins, once their existence is known.

There are two forms the test of the present invention can take. First, the actual hemolysin itself can be measured in serum or other bodily fluids (tracheal secretions, bronchiolar lavage fluid, nasal lavage, sinus tissues or secretions, etc.). In the second type of test, the antibodies (IgG, IgE, etc.) made by the exposed animal, after exposure to the fungus and the hemolysin it produces, are measured.

In the situation in which hemolysin *per se* is measured, the measurement can be effected by any type of conventional procedure for detecting proteins. These procedures include GC-MS, MALDI, ELISA, among others for quantifying proteins. In this case, the antibody is made by repeated injections of the hemolysin or fragments of the hemolysin into an animal such as a rabbit, and the antibodies produced by the rabbit are collected. These antibodies are then conjugated to any number of "reporting" molecules or enzymes. Any type of ELISA or other forum can be used for detection.

The second type of test actually measures the exposure of an animal to a hemolysin-producing fungus by measuring antibodies to the hemolysin produced by the animal.

In this case, purified hemolysin protein is used to capture the specific antibodies. The conventional sample is serum, but the antibodies can be detected in other bodily fluids such as nasal secretions. By measuring the antibodies to specific fungal hemolysins, the types of fungi to which the animal has been exposed can be detected. Detecting whether the antibody produced is IgG or IgE makes the test even more specific, since IgE is associated with allergies and asthma.

"Infection" is generally considered to be an invasive situation, *i.e.*, the organism itself actually penetrates the tissue of the host or causes, by its continuing presence, disease.

With many fungi, infection is thought not to occur. Rather, it is the allergens, toxins, etc., rather than the organism's continuing presence, that is thought to be the source of the health problem. Many microbiologists now consider that these fungi cause health problems by "colonizing" the sinuses or other susceptible parts of the body. Even though some species of fungi can be true infectious agents, they can be difficult to diagnose because of the lack of detectable antibodies or surface proteins.

Art Rejections

Claims 23 and 25-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sakaguchi et al. in view of Harlow et al.

This rejection is respectfully traversed. Sakaguchi et al. were not looking to see if the mouse was exposed to a fungus. Rather, Sakaguchi et al. were looking at the biological activity of hemolytic toxin from one species of fungus. Sakaguchi et al. themselves infected the mice, and then detected viable fungus in kidney and brain tissue. Secretion of *Asp.* hemolysin was observed immunohistochemically. There is no disclosure or even suggestion that this technique could be used to determine if a mammal has been exposed to a fungus, because Sakaguchi et al. injected the mice with the fungus to study the role of the toxin produced by the fungus.

There is nothing in Sakaguchi et al. that would lead one to believe that hemolysin proteins are produced by fungi and that species-specific hemolysin proteins (or antibodies thereto) would be detectable in bodily fluids. Nor would one skilled in the art reading Sakaguchi et al. be led to use other fungal hemolysins to measure the ambient exposure of an animal to a particular fungus, because Sakaguchi et al. specifically challenged mice by injection with only one

fungus. The samples Sakaguchi et al. used to detect the hemolysin brain, kidney, etc., are not samples one would use for detecting exposure to a pathogen. Sakaguchi et al. were studying the progress of fungal infection in mice, and thus were obtaining samples of tissues affected by the fungus. These types of samples are invasive and dangerous to a patient, and would not be used to detect exposure.

The Sakaguchi et al paper was published in 1984, yet no reliable test for fungi had been developed since. Those skilled in the art knew that fungi infested buildings where animals could be exposed to potentially harmful fungi, yet there was no test for these fungi. It was not until the present inventor's discovery that some specific fungi produce hemolysins that can be isolated and used for testing an animal's exposure to the fungi that an assay was available for testing for exposure to these fungi. The need for a reliable test for fungi had extended far beyond the publication of the Sakaguchi et al. paper in 1984, and there are currently prospective licensees for the present application.

As Judge Learned Hand stated in *Safety Car Heating & Lighting Co. v. General Electric Co*, 69 USPQ 401, 403 (2d Cir. 1946), "the length of time the art, though needing the invention, went without it" as the best non-technical guidepost for inferring obviousness. Judge Easterbrook noted

in *In re Mahurkar Patent Litigation*, 28 USPQ2d 1801 (N.D. Ill, 1993), *aff'd* 37 USPQ2d 1138 (Fed. Cir. 1995), "The existence of an enduring, unmet need is strong evidence that the invention is novel, not obvious, and not anticipated. If people are clamoring for a solution, and the best minds do not find it for years, that is practical evidence—the kind that can't be bought from a hired expert, the kind that does not depend on fallible memories or doubtful inferences—of the state of knowledge."

Harlow et al. add nothing to this disclosure, because Harlow et al. merely teach direct labeling of antibodies in immunohistochemical techniques, that is, an alternative method for detecting antibodies.

The present invention is not directed to a specific method for detecting hemolysin. The present invention is not directed to a specific immunohistochemical method. The present invention is directed to a method for determining if an animal or a building has been exposed to a specific hemolysin-producing fungus.

As described above, the present inventor has discovered that there are a number of fungi that produce hemolysins specific to each fungus, and he has discovered how to obtain these hemolysins for use in assays for detecting the presence of these fungi. This is particularly important,

because for many fungi, such as *Stachybotyrus chartarum*, normal antibody production to the body or mycelium of the organism does not occur in humans. However, the present inventor has discovered that many fungi do produce hemolysins, and has developed a method for recovering these hemolysins for use in assays. As stated in the last sentence of paragraph 0021 of the instant specification, the assay can be of any conventional immunoassay type, such as ELISA, RIA, etc. Thus, it is not the specific type of immunoassay that is claimed herein, but, rather, a method for determining if an animal has been exposed to a particular hemolysin-producing fungus.

Paragraph 0032 of the specification as filed states, "These antibodies to fungal hemolysin can be used in a conventional immunoassay such as an ELISA to determine if one has been exposed to strains of fungi which produce hemolysin. The hemolysin protein itself can be used to determine if an animal has produced antibodies in response to exposure to the fungus.

Specification

The specification is objected to as failing to provide proper antecedent basis for the claimed subject mater. The Examiner alleges that the specification fails to provide written description support for the term "specific hemolysin-producing fungus."

Attention is directed to paragraph 0033 on page 8 of the specification as filed, "The present invention thus provides a method to determine if a human or other animal has been exposed to a hemolysin-producing fungus, such as *Stachybotyrus chartarum*. By assaying samples from a human or other animal for antibodies to a hemolysin-producing fungus, it is now possible to determine if the human or other animal has been exposed to such a fungus."

It is clear that this paragraph provides support for a "specific hemolysin-producing fungus", because "a hemolysin-producing fungus, such as *S. chartarum*" is a specific hemolysin-producing fungus. By assaying samples for antibodies to a hemolysin-producing fungus, one can determine if the animal has been exposed to a fungus; that is, the antibodies are specific to that fungus, so one can assay a sample for a particular fungus.

As the Examiner is well aware, an objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 10 USPQ2d 1614 (Fed. Cir. 1989). As MPEP 2163.02 provides, "The subject matter of the claim need not be described literally (*i.e.*, using the same

terms or *in haec verba*) in order for the disclosure to satisfy the description requirement.

In the present case, as one skilled in art can readily ascertain, a specific hemolysin-producing fungus is *S. chartarum* or any of the other specific hemolysin-producing fungi described in the instant specification. Therefore, there is antecedent basis in the specification for "specific hemolysin-producing fungus." If the fungus did not produce a hemolysin specific to that fungus, one would not be able to assay for a specific fungus, such as *S. chartarum*, or any other particular fungus, only that there had been exposure to some type of fungus.

One skilled in the art, knowing that there is a hemolysin for a fungus of interest, can then detect this hemolysin by conventional methods. Any microbiologist is aware of the existence of **bacterial** hemolysins, which have been studied for more than 30 years. These are very important proteins produced by bacteria. Therefore, any microbiologist would know how to examine the bacterial scientific literature and apply the methods of producing and using bacterial hemolysins to produce and use fungal hemolysins.

Claim 33 is rejected under 35 U.S.C. 102(b) as being anticipated by Sakaguchi et al.

This rejection is respectfully traversed. There is nothing in Sakaguchi et al. that would lead one skilled in the art to use a hemolysin for detecting exposure to a fungus. Sakaguchi et al. knew that the animal had been exposed to a hemolysin-producing fungus because they injected the animal with the fungus. Sakaguchi et al. were interested in the role of hemolytic toxin and its biological activity during experimental *Aspergillus* infection in mice. Sakaguchi et al. assayed samples from kidney, heart, liver, and brain, which samples were undoubtedly obtained from sacrificed mice. There is nothing at all in Sakaguchi et al. regarding determining if an animal has been exposed to the fungus. Sakaguchi et al. were merely looking to see the effects of the fungus on the mice, as well as whether antitoxin would be effective against the fungus. There is no recognition in Sakaguchi et al. that a specific hemolysin could be used to identify each fungus, or that hemolysin could be used to detect exposure to the fungus.

Claims 23-29 and 33 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Examiner alleges that there is no basis in the specification for claming "a specific hemolysin-producing fungus."

This rejection is respectfully traversed. The objects of the invention, at paragraphs 0012 and 0013 state that it is an object of the present invention to provide a method and reagent for screening humans and other animals for exposure to hemolysin-producing fungi. It is another object of the present invention to provide a method and reagent for screening humans and other animals for exposure to *Stachybotyrus chartarum*. Paragraph 0015 states that it is a further object of the present invention to identify strains of fungi using an *in vitro* test. Paragraph 0024 states, "By growing strains of hemolysin producing fungi *in vitro* and isolating the hemolysin, it is now possible to use the protein obtained to identify fungi which are isolated from buildings, homes, schools, and the like."

One cannot identify strains of fungi as stated in paragraph 0015 unless there is a distinct marker for each fungus, namely, a specific marker, or a marker specific to each fungus. Therefore, one skilled in the art reading the present specification would appreciate that each fungus had a specific hemolysin which can be used to identify the fungus.

In describing diagnosis of infection with *S. Chartarum*, beginning at paragraph 0025 on page 6, hemolysin was obtained from the fungus. In the Diagnosis section, beginning at paragraph 0028 on page 7, the specification

states in paragraph 0030, "The hyperimmune serum of rabbits immunized with the KLH-protein was processed over the above immunosorbent to capture antibodies **specific for the fungal hemolysin protein** [emphasis added]." Using these specific antibodies, the present invention thus provides a method to determine if a human or other animal has been exposed to a hemolysin-producing fungus such as *S. chartarum*.

One skilled in the art reading the present specification could readily ascertain that the hemolysin is specific to a particular fungus, and only the hemolysin-producing fungi can be detected in the herein claimed manner.

Claims 30-32 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Examiner states that claims to determining if a building contains a hemolysin-producing fungus constitutes new matter.

This rejection is respectfully traversed. The level of skill of one skilled in this particular art is relatively high, *i.e.*, a skilled microbiologist. Therefore, one would expect that one skilled in this art could readily extrapolate, without undue experimentation, a method for detecting the fungi isolated from a building suspected of containing the hemolysin-producing fungi. The present specification provides ample directions for detecting hemolysin using antibodies to

the specific hemolysin, specifically at paragraph 0025 through paragraph 0033. The invention relates to using specific hemolysin for detecting a specific fungus. Once one skilled in the art appreciates that each hemolysin-producing fungus has its own specific hemolysin, an assay can be derived for each hemolysin from known techniques for detecting proteins.

Claims 30-32 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner alleges that the claims contain subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains or with which it is most nearly connected, to make and/or use the invention.

This rejection is respectfully traversed. Claims 30-32 have been amended to recite that the sample is cultured so that any hemolysin-producing fungus will produce the hemolysin, which can then be detected. One skilled in the art, knowing methods for assaying proteins, can readily derive an assay method based upon conventional methods, using the hemolysin or antibodies to the hemolysin, from a specific fungus

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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